UNIVERSITY OF CALIFORNIA, SAN DIEGO

Mechano-Chemical Model of Cancer Cell Invasion

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by

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2013
The thesis of Jui-Hsien Wang is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

2013
DEDICATION

To my parents, Ming-Fang and Shu-Hua, and to Emma.
If nature were not beautiful it would not be worth knowing,
and life would not be worth living.

—Henri Poincaré
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90% of the cancer-inflicted mortality comes from the acquired metastatic ability. Metastatic cancer cells can physically travel through the extracellular matrix (ECM) by regulating enzymatic proteolysis. By colocalizing enzymes such as Matrix Metalloproteases (MMPs) at the invasion site, cancer cells actively degrade the ECM structures to promote the invasion efficiency. In this theoretical study, we propose a mechano-chemically coupled model to study this multiphysical phenomenon. We use the regular perturbation method to derive an analytical
expression for the deformation field induced by cell-exert stresses on a nonlinear, inhomogeneous material. We then propose a chemical model that reflects the dynamics of the system, which includes the enzyme kinetics and enzyme diffusion. The mechanical and chemical models are coupled under the considerations that, (1) material stiffness is related to the concentration of the denaturized substrate constituent; (2) catalytic reaction is accelerated by the mechanical strain energy, as found in the recent single molecule assays. Using this model, we study a synthetic stress field that is consistent with the experimental observations. It is found that by coupling force to the degradation process, the dynamics of the system is significantly altered. We perform a parametrized study on mechanical forces, colocalized enzyme concentration, and the enzyme release pattern to study its individual effects on the invasion. Qualitatively, the study reveals the importance of each parameters on affecting the invasion efficiency from a rigorous physical and mathematical point of views.
Chapter 1

Introduction

Three dimensional cell migration and tissue invasion is central to many biological processes, such as embryogenesis, tissue morphogenesis, immune system responses, and cancer progression. A key component in cancer progression is its acquired metastatic ability to travel through the extracellular matrix (ECM), enter blood or lymphatic vessels and land on the distant site where it can grow a secondary tumor. In fact, as reported 90% of the cancer-inflicted mortality comes from this physical translocation [1, 2]. Despite its importance, a large portion of this process remains unclear.

Cancer cell invasion is in nature a fascinating multiphysical process. As the invasion can be interpreted as the mechanical deformation induced by the cell-exert forces, cancer cell can perform delicate tricks chemically to accelerate this process. For example, depending on the underlying ECM stiffness, cancer cell is capable of switching between protease-independent amoeboid invasion mode [3] and protease-dependent mesenchymal invasion mode [4, 5, 6]. The latter is characterized by the elongated cell morphology, with strong adhesion to the substrate and the capability to secrete and activate a particular family of enzyme, matrix metalloproteases (MMPs), to actively remodel the underlying substratum [7, 5, 8, 9]. Moreover, the activation of MMPs is shown to be closely associated with integrins [5, 10, 11, 12], implying that ECM proteolysis could be triggered by force generation at the focal adhesion. Once activated, the stiffness of the ECM barrier is
lowered and the invasion is promoted [13]. On the other hand, mechanical strain has a feed-back effect on the degradation rate, too. Bulk measurements on the collagen network show that mechanical strain protects the fibril composing the ECM structure [8, 14, 15, 16], while latest molecule assays reveals otherwise, that mechanical strain alters the molecule conformation and accelerate the degradation [17, 18]. One way or another, this is a mechano-chemically coupled problem and model with rigorous mathematical and physical basis is needed to understand the process in the theoretical point of view.

In this study, we model the following situation. The cell is initially adhered to the surface of an elastic, thick substratum with base Young’s modulus, $E_0$, and Possion ratio, $\sigma$. At time zero, the cell instantaneously forms adhesion sites, generates a traction stress field on the surface and begins to invade vertically into the substratum; meanwhile, relevant enzymes are released on the substratum surface with a known distribution and conserved total concentration. Adapted from the experiment performed by Aung et al. [19], FIG. 1.1 illustrates the colocalization of F-actin and MT1-MMP at the leading edge of a breast cancer cell when it is invading to the Matrigel network, validating the geometrical configuration of the model shown in FIG. 1.2.

![Figure 1.1](image)

Figure 1.1: Confocal images showing the colocalization of F-actin and MT1-MMP at the leading edge of a breast cancer cell when it is invading to the Matrigel network (cell line: MDA-MB-231). Images adapted from Aung et al. [19].
The input data are the synthetic three direction stresses exerted by the cell on the free surface of the substratum ($z = 0$, blue), and it is assumed that the deformation of the substratum is zero at the bottom surface ($z = -h$, red). For simplicity semi-infinite material depth is considered and therefore $h \to \infty$. We assume that the substratum has base Young’s modulus $E_0$ and constant Poisson’s ratio $\sigma$. Fourier series with spatial periods $L$ and $W$ are used to respectively express the dependence of the variables in the $x$ and $y$ direction.

The modeling paradigm is illustrated in FIG. 1.3 as a mechano-chemical feedback system. For the mechanical part, we modify the Fourier traction force cytometry method (TFM), similar to del Álamo et al. [20, 21]. We employ regular perturbation expansion to the Young’s modulus, which characterizes the stiffness of a material, to account for the nonlinearity and inhomogeneity due to enzymatical degradations [22]. To integrate the chemical reaction and study the dynamics of the system, we formulate a reaction-diffusion equation that can be solved numerically to represent the time evolution of enzymatic degradation. Finally the two parts are coupled to form the mechano-chemical model of this study. This model is used to study a synthetic force field that is consistent with experimental observations, and to perform a parametrized study. The model construction is in chapter 2 (mechanical part) and chapter 3 (chemical part); the analysis is in
Chapter 4.

![Diagram of feedback system of mechano-chemical model](image)

Figure 1.3: Illustration of the feedback system of the mechano-chemical model.

Chapter 1, in part, is currently being prepared for submission for publication of the material. Jui-Hsien Wang; Juan Carlos del Álamo. The thesis author was the primary investigator and author of this material.
Chapter 2

Elastostatic Problem of Nonlinear, Inhomogeneous Substrata

2.1 Problem formulation

We consider the cell moving on the surface of an elastic three-dimensional substratum with base Young’s modulus $E_0$ and Poisson’s ratio $\sigma$. The adhering cell induces traction forces on the substratum surface, which is described by functions $\tau_{zz}(x, y)$, $\tau_{zy}(x, y)$, and $\tau_{zz}(x, y)$ on the surface. The bottom of the substratum is assumed to be in contact with a stiff surface and therefore the no-slip condition is applied. For simplicity, the substratum is considered to be thick compared with the cell length. FIG 1.2 sketches this configuration.

The negligible mass and the slow characteristic speed of the migrating cell allow us to model the system using elasticity equation in equilibrium,

$$\nabla \cdot \tau = 0,$$  \hspace{1cm} (2.1)

where $\tau$ is the stress tensor acting on the substratum. This assumption is justified by estimating the ratio between the inertial and elastic stresses, which is of order $\rho l^2/E_0 T^2 \approx 10^{-12}$, where $\rho \sim 10^3 kg \cdot m^{-3}$ and $E_0 \sim 10^3 Pa$ are respectively the
density and base Young’s modulus of the substratum, \( l \sim 2 \times 10^{-5} \text{m} \) is the length of the cell and \( T \sim 20 \text{s} \) is the characteristic timescale of pseudopod protrusion and retraction [23]. The stress can be expressed as a function of strain for a linear material by Hooke’s law,

\[
\tau_{ik} = \frac{E}{1 + \sigma} (u_{ik} + \frac{\sigma}{1 - 2\sigma} u_{il} \delta_{ik}),
\]

(2.2)

\( E \) is the Young’s modulus and \( u_{ik} = \frac{1}{2} (\frac{\partial u_i}{\partial x_k} + \frac{\partial u_k}{\partial x_i}) \) is the strain tensor. We model the weakly nonlinear, inhomogeneous Young’s modulus using regular perturbation theory,

\[
E = E_0 + \epsilon E_1 = E_0 [1 + \epsilon f(x, y, z)],
\]

(2.3)

where \( f(x, y, z) \) is an arbitrary function and \( \epsilon \ll 1 \) is the perturbation parameter. This general representation is kept throughout the calculation so it can be used to study different problems. For example, by making \( f(x, y, z) = x \), we can describe a material with linear stiffness gradient in the \( x \)-direction; by making \( f(x, y, z) \) depends on mechanical strain, we can describe a material with stress-stiffening (e.g. in vivo cross-linked Collagen network) or stress-softening (e.g. in vitro Matrigel) effect, depending solely on the sign of \( f(x, y, z) \). The formulation is valid as long as \( f(x, y, z) \sim O(1) \).

The displacement field \( \mathbf{u} = (u, v, w) \) must also be expanded by \( \mathbf{u} = \mathbf{u}_0 + \epsilon \mathbf{u}_1 \). Consequently, we can express the governing equations of the deformation field as

\[
\nabla (\nabla \cdot \mathbf{u}_0) \frac{1}{1 - 2\sigma} + \nabla^2 \mathbf{u}_0 = 0
\]

(2.4)

in the zeroth order and

\[
\nabla (\nabla \cdot \mathbf{u}_1) \frac{1}{1 - 2\sigma} + \nabla^2 \mathbf{u}_1 = -\frac{2(1 + \sigma)}{E_0} \nabla \cdot [f(x, y, z) \bar{\mathbf{f}}_0]
\]

(2.5)

in the first order.
2.2 Analytical solution

2.2.1 Zeroth order solution

The leading order is a forward problem whose fundamental solutions were obtained by Boussinesq [24]. The inverse counterpart of the problem (i.e. given deformation to find stress) was solved by Butler et al. for infinite thickness material for two-dimensional deformation field [25] and del Álamo et al. for finite thickness material for both two- [20] and three-dimensional deformation [21]. We follow the more general formulation by del Álamo et al, which can be easily extended to the first order solution. We seek for solutions of the leading order equation (2.4) that are periodic in both horizontal directions and expressed them using a Fourier series,

\[
\mathbf{u}_0 = \sum_{m=-\infty}^{\infty} \sum_{n=-\infty}^{\infty} \hat{\mathbf{u}}_{0,mn}(z)e^{i\alpha_m x + i\beta_n y}, \tag{2.6}
\]

where \(\alpha_m = 2\pi m/L\), \(\beta_n = 2\pi n/W\) are respectively the wavenumbers in the \(x\) and \(y\) directions. For simplicity, we will drop the subindices \(m\) and \(n\) of \(\alpha\) and \(\beta\) in what follows.

The Fourier coefficient vector \(\hat{\mathbf{u}}_{0,mn}\) can be expressed in the form

\[
\hat{\mathbf{u}}_{0,mn}(z) = [\mathbf{U}_{mn}(z) | \mathbf{V}_{mn}(z) | \mathbf{W}_{mn}(z)] \cdot d_z \hat{\mathbf{u}}_{0,mn}^h = \mathbf{U}_{mn}(z) \cdot d_z \hat{\mathbf{u}}_{0,mn}, \tag{2.7}
\]

where \(\mathbf{U}_{mn}(z)\), \(\mathbf{V}_{mn}(z)\), and \(\mathbf{W}_{mn}(z)\) are the three fundamental solutions of the problem. They were given explicitly by del Álamo et al. [20, 21] and were confirmed here again. Please refer to Appendix A for the explicit expression of the fundamental solutions. Note that it has slightly different form compared with these papers because of the different coordinate systems. It is simply shifted by the amount \(z \rightarrow z + h\), so that it remains zero at the lower bottom. \(\mathbf{U}_{mn}(z)\) is the resolvent matrix and \(d_z \hat{\mathbf{u}}_{0,mn}^h\) is the \(z\)-derivative of \(\hat{\mathbf{u}}_{0,mn}\) at \(z = -h\) where the no-slip condition is applied, i.e. \(\mathbf{u}_0(x, y, -h) = 0\). \(d_z \hat{\mathbf{u}}_{0,mn}^h\) is unknown \textit{a priori}. 
We solve the unknown coefficients using the prescribed stresses on the free surface

\[ \tau_{0,zz}(z = 0) = \tau_{zz}^0, \quad (2.8) \]
\[ \tau_{0,zx}(z = 0) = \tau_{zx}^0, \quad (2.9) \]
\[ \tau_{0,zy}(z = 0) = \tau_{zy}^0. \quad (2.10) \]

Here subscript 0 indicates the zeroth order solution and the superscript 0 indicates the boundary conditions on the surface. The stress and strain are related by applying Hooke’s law in the Fourier space,

\[ \hat{\tau}_{0,mn}(z) = \begin{bmatrix} \hat{\tau}_{0,xx}(z) \\ \hat{\tau}_{0,xy}(z) \\ \hat{\tau}_{0,xz}(z) \end{bmatrix}_{mn} = \mathcal{H}_{mn} \cdot \begin{bmatrix} \hat{u}_{0,mn}(z) \\ d_z \hat{u}_{0,mn}(z) \end{bmatrix}. \quad (2.11) \]

\( \mathcal{H}_{mn} \) is a 3-by-6 Hooke’s matrix which depends on the Poisson ratio, \( \sigma \), base Young’s modulus, \( E_0 \), and the wavenumbers (see Appendix A). \( d_z \hat{u}_{0,mn} \) is the analytical differentiation of the Fourier coefficient \( \hat{u}_{0,mn} \). Particularizing this relation on the surface, we express the unknowns in terms of the vertical stresses exerted on the surface.

\[ \hat{\tau}_{mn}^0 = \mathcal{A}_{mn} \cdot d_z \hat{u}_{0,mn}^h, \quad \mathcal{A}_{mn} = \mathcal{H}_{mn} \cdot \begin{bmatrix} U_{mn}(0) \\ d_z U_{mn}(0) \end{bmatrix}. \quad (2.12) \]

Substitute this relation back to Eq 2.7, the deformation vector is therefore

\[ \hat{u}_{0,mn}(z) = \hat{G}_{mn}(z) \cdot \hat{\tau}_{mn}^0 = U_{mn}(z) \cdot \mathcal{A}_{mn}^{-1} \cdot \hat{\tau}_{mn}^0, \quad (2.13) \]

where the Green’s function \( \hat{G}_{mn}(z) \) in the Fourier space is given explicitly in terms of wavenumber \( \alpha, \beta, \) and \( k = (\alpha^2 + \beta^2)^{1/2} \),

\[
\hat{G}_{mn}(z) = \frac{(1 + \sigma) e^{kz}}{E_0} \begin{bmatrix}
\frac{\alpha^2 z k - 2 \alpha^2 \sigma + 2 k^2}{k^3} & \frac{\alpha \beta (-2 \sigma + k z)}{k^3} & \frac{i \alpha (-1 + 2 \sigma + k z)}{k^3} \\
\frac{-i \alpha (2 \sigma - 1 + k z)}{k^3} & \frac{\beta^2 z k - 2 \beta^2 \sigma + 2 k^2}{k^3} & \frac{-i \beta (2 \sigma - 1 + k z)}{k^3} \\
\frac{-i \alpha (2 \sigma + k z)}{k^3} & \frac{-i \beta (2 \sigma + k z)}{k^3} & \frac{2 - 2 \sigma - k z}{k}
\end{bmatrix}.
\]
2.2.2 First order solution

In the first order (\(\epsilon\)-order), the bottom of the substrate is subjected to the no-slip condition, i.e. \(\mathbf{u}_1(x, y, -h) = 0\). Recalled that we applied perturbation on Hooke’s law, which suggests that the stress is bilinear on Young’s modulus and deformation, the expanded form of the stress is

\[
\mathbf{\tau}(\mathbf{u}; E) = \mathbf{\tau}(\mathbf{u}_0; E_0) + \epsilon[\mathbf{\tau}(\mathbf{u}_1; E_0) + \mathbf{\tau}(\mathbf{u}_0; E_1)],
\]

where \(\mathbf{\tau}(\mathbf{u}_0; E_1) = f(x, y, z) \cdot \mathbf{\tau}(\mathbf{u}_0; E_0)\), inferred again by the linearity of Hooke’s law. The Fourier representation of the first order surface stress is therefore

\[
\mathbf{\tau}_{1,xx}(z = 0) = \mathbf{\tau}_{xx}^{1},
\]

\[
\mathbf{\tau}_{1,zy}(z = 0) = \mathbf{\tau}_{zy}^{1},
\]

\[
\mathbf{\tau}_{1,zz}(z = 0) = \mathbf{\tau}_{zz}^{1},
\]

where \(\mathbf{\tau}^1 = -f \cdot \mathbf{\tau}^0\) is the altered forcing in the first order, particularized on the surface. We thus define the complementary forcing \(\mathbf{F}(x, y, z) = f(x, y, z) \cdot \mathbf{\tau}_0\) for future reference. The formulation enforces the equilibrium constraint of the underlying elastostatic equation by introducing an additional forcing in the first order, i.e. the extra force field induced by the gradient of the Young’s modulus in the zeroth order can be transferred to the first order problem and balanced out accordingly. Because of this compatibility relation, the first order deformation can have nonzero average, unlike the zeroth order solution on a linear material. We will discuss this matter later.

We exploit the linearity of the first order equation and pursue the solutions to two complementary problems: 1) \(\mathcal{L}(\mathbf{u}_{1h}) = 0\) with boundary conditions \(\mathcal{H}(\mathbf{u}_{1h})|_{z=0} = \mathbf{\tau}^1(x, y)\) and \(\mathbf{u}_{1h}|_{z=-h} = \mathbf{\zeta}(x, y)\). \(\mathbf{\zeta}\) is the altered Dirichlet boundary condition at the bottom, which will be defined below. We slightly abuse the notation here and use \(\mathcal{H}(\cdot)\) to indicate the Hooke’s operator with base Young’s modulus \(E_0\); \(\mathcal{L}(\cdot)\) is the first order elastostatic equation operator. 2) \(\mathcal{L}(\mathbf{u}_{1p}) = \mathbf{\xi}\), where \(\mathbf{\xi} = -2(1+\sigma)\nabla \cdot \mathbf{F}/E_0\) is the body force term of the first order equation 2.5. \(\mathbf{u}_{1p}\) has homogeneous stress boundary condition on the free surface. The solution
to the first order, $u_1$, is the superposition of the two: $u_1 = u_{1h} + u_{1p}$.

The inhomogeneous solution, $u_{1p}$, accounts for the forcing of the equation and can be sought using variation of parameters or other similar technique, as the fundamental solution is known to the homogeneous counterpart of the equation. To utilize this advantage, we again pose the problem to the Fourier domain and seek $u_{1p}$ of the form

$$u_{1p}(x, y, z) = \sum_{m=-\infty}^{\infty} \sum_{n=-\infty}^{\infty} \hat{u}_{1p, mn}(z) e^{i\alpha mx + i\beta ny},$$

(2.19)

associated with the complementary forcing $F(x, y, z)$ of the form

$$F(x, y, z) = \sum_{m=-\infty}^{\infty} \sum_{n=-\infty}^{\infty} \hat{F}_{mn}(z) e^{i\alpha mx + i\beta ny}.$$  

(2.20)

The Fourier coefficients of the body force term $\hat{\xi}_{mn} = [\hat{\xi}_x, \hat{\xi}_y, \hat{\xi}_z]^T_{mn}$ can therefore be written as the linear superposition of components of $F$. Finally, $u_{1p}$ can be formulated by the integral

$$\hat{u}_{1p, mn}(z) = [\mathcal{U}_{mn}^*(z) | \mathcal{U}_{mn}(z)] \int_0^z \left[ \frac{\mathcal{U}_{mn}(s)}{d_s \mathcal{U}_{mn}^*(s)} \right]^{-1} \left[ \begin{array}{c} 0 \\ \hat{\xi}_{mn}(s) \end{array} \right] ds,$$

(2.21)

where $\mathcal{U}_{mn}(z) = [\mathcal{U}_{mn}^*(z) | \mathcal{V}_{mn}(z) | \mathcal{W}_{mn}(z)]$ is the complementary fundamental solution given in Appendix A; $d_s \mathcal{U}_{mn}^*(z)$ is the analytical differentiation of $\mathcal{U}_{mn}^*(z)$; $\mathcal{U}_{mn}(z)$ and $d_s \mathcal{U}_{mn}(z)$ are the fundamental solution defined in the previous sections (same as zeroth order). $\boldsymbol{\xi}(z)$ is the nonlinear product between $\mathbf{f}$ and $\mathbf{\tau}_0$. This nonlinear product is a convolution integral in the Fourier domain, and thus pseudo-spectral method is utilized to avoid the potential computation burden when calculating $\hat{\xi}_{mn}$.

The homogeneous part of the first order solution, $u_{1h}$, is characterized by an identical governing equation $\mathcal{L}(u_{1h}) = 0$ as in the zeroth order. However, the boundary condition at the bottom is no longer trivial because $u_{1p}$ is in general
not zero at the lower boundary, and \( u_1(x, y, -h) = u_{1p}(x, y, -h) + u_{1h}(x, y, -h) = 0 \). To satisfy the original boundary conditions, at the bottom of the substratum \( u_{1h}|_{z=-h} = \zeta(x, y) = -u_{1p}(z = -h) \). Similarly, we seek the solution in the Fourier domain

\[
u_{1h}(x, y, z) = \sum_{m=-\infty}^{\infty} \sum_{n=-\infty}^{\infty} \hat{u}_{1h,mn}(z) e^{i\alpha_m x + i\beta_n y}, \quad (2.22)
\]

the Fourier coefficient \( \hat{u}_{1h,mn}(z) \) of which can be written as

\[
\hat{u}_{1h,mn}(z) = \left[ U^*_{mn}(z) | U_{mn}(z) \right] \cdot \begin{bmatrix} \zeta \\ d_z \hat{u}^{-h}_{1,mn} \end{bmatrix}, \quad (2.23)
\]

where \( d_z \hat{u}^{-h}_{1,mn} \) is the unknown \( z \)-derivative of \( \hat{u}^{-h}_{1,mn} \) particularized at \( z = -h \). \( \zeta \) is a known quantity and can be calculated with the knowledge of the Young’s modulus distribution (and thus the body force \( \zeta \)).

A representation of the first order solution \( \hat{u}_{1,mn}(z) \) and the associated \( d_z \hat{u}_{1,mn}(z) \) can then be formulated by combining the two solutions

\[
\hat{u}_{1,mn}(z) = \hat{u}_{1h,mn}(z) + \hat{u}_{1p,mn}(z), \quad (2.24)
\]

\[
d_z \hat{u}_{1,mn}(z) = d_z \hat{u}_{1h,mn}(z) + d_z \hat{u}_{1p,mn}(z), \quad (2.25)
\]

where the derivatives are given analytically as

\[
d_z \hat{u}_{1h,mn}(z) = \left[ d_z U^*_{mn}(z) | d_z U_{mn}(z) \right] \cdot \begin{bmatrix} \zeta \\ d_z \hat{u}^{-h}_{1,mn} \end{bmatrix}, \quad (2.26)
\]

\[
d_z \hat{u}_{1p,mn}(z) = \left[ d_z U^*_{mn}(z) | d_z U_{mn}(z) \right] \int^z_0 \begin{bmatrix} U^*_{mn}(s) & U_{mn}(s) \\ d_z U^*_{mn}(s) & d_z U_{mn}(s) \end{bmatrix}^{-1} \begin{bmatrix} 0 \\ \xi(s) \end{bmatrix} ds. \quad (2.27)
\]

The first order stresses are defined from the first order deformation acting on an unperturbed material. To relate the stress and strain, we apply Hooke’s law to the
solution vector like we did for zeroth order problem

\[
\hat{\tau}_{1,mn}(z) = \begin{bmatrix} \hat{\tau}_{1,zx}(z) \\ \hat{\tau}_{1,zy}(z) \\ \hat{\tau}_{1,zz}(z) \end{bmatrix}_{mn} = \mathcal{H}_{mn} \begin{bmatrix} \hat{u}_{1h,mn}(z) \\ \frac{d_z \hat{u}_{1h,mn}(z)}{d_z} \end{bmatrix}_{mn} + \mathcal{H}_{mn} \begin{bmatrix} \hat{u}_{1p,mn}(z) \\ \frac{d_z \hat{u}_{1p,mn}(z)}{d_z} \end{bmatrix}_{mn}.
\]  

(2.28)

On the free surface, \( z = 0 \), the stress induced by \( \hat{u}_{1p,mn} \) is zero as required by the boundary condition and the only non-zero contribution comes from \( \hat{u}_{1h,mn} \).

\[
\hat{\tau}^1_{mn} = \mathcal{A}_{mn} \cdot d_z \hat{u}^{-h}_{1,mn} + \mathcal{A}^*_{mn} \cdot \zeta. 
\]  

(2.29)

\( \mathcal{A}_{mn} \) was defined in previous sections and \( \mathcal{A}^*_{mn} = \mathcal{H}_{mn}[\mathcal{U}^*_{mn}(0), d_z \mathcal{U}^*_{mn}(0)]^T \) is an additional contribution coming from the forcing of the first order equation. This expression closes the system. When the substrate thickness is much larger than the regions of interest, \( z/h \ll 1 \), the first order deformation of the system can be represented by the simple equation

\[
\hat{u}_{1,mn}(z) = \hat{G}_{mn}(z) \cdot \hat{\tau}^1 + \hat{G}^*_{mn}(z) \cdot \zeta + \hat{u}_{1p,mn}(z).
\]  

(2.30)

\( \hat{G}_{mn}(z) \) was given in equation 2.14 and \( \hat{u}_{1p,mn}(z) \) was given in equation 2.21, and

\[
\hat{G}^*_{mn}(z) = \mathcal{U}^*_{mn}(z) - \mathcal{U}_{mn}(z) \cdot \mathcal{A}^{-1}_{mn} \cdot \mathcal{A}^*_{mn}.
\]  

(2.31)

is the complementary Green’s function to the first order problem (refer to Appendix A). Both the complementary Green’s function and particular solution \( \hat{u}_{1p,mn} \) vanishes at the free surface as the substratum thickness approaches infinity \( kh \to \infty \), and the solution depends solely on the stress boundary condition (the first term in Eq. 2.30), as expected from physical intuitions.

### 2.2.3 Zeroth Fourier Mode of the first order solution

Notice that the zeroth order solution as obtained in Eq. 2.13 and the first order solution as obtained in Eq. 2.31 are both singular at the zeroth Fourier mode,
when $\alpha = \beta = 0$. As this problem is invariant up to a constant, we need to consider the averaged first order equation to get the correct representation of the first order solution at the zeroth Fourier mode (zeroth order deformation is constrained to have zero average because the solution has to be in equilibrium in an unperturbed case). We study the zeroth Fourier mode of the first order equation by substituting $\alpha = \beta = 0$ into Eq. 2.5:

$$
\begin{align*}
\frac{d}{dz} \begin{pmatrix}
\tilde{u}_1(z) \\
\tilde{v}_1(z) \\
\tilde{w}_1(z)
\end{pmatrix} + \frac{1}{1 - 2\sigma} \frac{d}{dz} \begin{pmatrix}
0 \\
0 \\
\tilde{w}_1(z)
\end{pmatrix} = -\frac{2(1 + \sigma)}{E_0} d_z \begin{pmatrix}
\tilde{F}_{zz}(z) \\
\tilde{F}_{zy}(z) \\
\tilde{F}_{zz}(z)
\end{pmatrix},
\end{align*}
$$

(2.32)

where tilde represents the Fourier coefficient of that quantity in the zeroth mode $\tilde{\cdot} = (\cdot)_{0,0}$. This system is subjected to no-slip condition at the bottom and the averaged first order stress $\langle \mathbf{\tau}_1 \rangle = -\langle \mathbf{F}(x, y, 0) \rangle$ at the surface. This can be re-written as a system of first order linear differential equations and its solution is given by the integral

$$
\begin{align*}
\begin{pmatrix}
\tilde{u}_1(z) \\
\tilde{v}_1(z) \\
\tilde{w}_1(z)
\end{pmatrix} = -\frac{2(1 + \sigma)}{E_0} \int_{-h}^{z} \begin{pmatrix}
\tilde{F}_{zz}(s) \\
\tilde{F}_{zy}(s) \\
\frac{1}{2 - 2\sigma} \tilde{F}_{zz}(s)
\end{pmatrix} ds.
\end{align*}
$$

(2.33)

This result suggests that if the averaged complementary forcing, $\langle \mathbf{F} \rangle$, is not zero, the average of the first order deformation will also be non-zero. Its magnitude will be proportional to the overall complementary forcing in the z-direction. This result also suggests that any non-zero averaged deformation field measured in the experiments might be due to the heterogeneity in the material properties integrated in the vertical direction; this is in constrast to the homogeneous, linear case where the forcing term is zero.

Chapter 2, in part, is currently being prepared for submission for publication of the material. Jui-Hsien Wang; Juan Carlos del Álamo. The thesis author was the primary investigator and author of this material.
Chapter 3

Mechano-chemical Model

3.1 Geometry

In the previous chapter, we developed a method to characterize the deformation field when the cell adheres to a substratum with nonlinear, inhomogeneous material properties. This technique is now used to study a model problem which mimics the essential features of the metastatic cancer cell invasion. Under the framework of perturbation method and small strain theory, the result from last chapter is rigorously appropriate on understanding the onset of this process.

Illustration of the model system is similar to FIG 1.2 with the model cell adhered at the center of the coordinate system. At $t = 0$, it instantaneously generates a traction force field on the surface and starts to invade downward, in the negative $z$-direction. At the same time, the model cell is able to secrete and activate a relevant enzyme to degrade the material, leading to inhomogeneous (and potentially nonlinear) material properties, especially around the cell where the enzyme concentration is the highest. This setup is of particular interest as recent experiments suggest that the cancer cell switches to proteases-dependent invasion mode when encountering stiff ECM structures [5, 12].
3.2 Dynamics

3.2.1 Enzyme kinetics

Because of the negligible inertia of the system, the time dependence of the problem comes from the kinetics of the chemical reaction, and the diffusion of chemical species. We use the Michaelis-Menten type kinetics to model the chemical reaction [26]. It is one of the most popular kinetic model to characterize the enzymatic proteolysis pertains to cancer cell invasion. Previous experiments conducted on collagen proteolysis [27, 17, 18], elastin proteolysis [28], and computational modeling of the enzymatic proteolysis [29] suggest that this kinetic scheme is capable of capturing the essential features of this process. The two-step reaction model is

\[
M + S \xrightarrow{k_1} C \xrightarrow{k_2} M + P,
\]

where \(M, S, C, P\) are respectively the concentration of the enzyme (e.g. matrix metalloproteases), substrate (e.g. collagen structure), enzyme-substrate complex, and the product (e.g. denaturized collagen fibril). \(k_1, k_{-1}, k_2\) are respectively the rate constants for the association, dissociation, and catalytic reactions. The reverse reaction of the second step \((k_{-2})\) is assumed to be unimportant [26].

3.2.2 Enzyme diffusion

The diffusion of the the chemical species introduces additional time scales. Substrate, complex, and the product are considered to be immobile; the enzyme is subjected to molecular diffusion whose magnitude can be estimated by its molecular weight. We note that, however, the enzyme-substrate complex may also have diffusive property as found for some enzyme-substrate combinations [30, 31]. Although our model is general enough to include these properties, for simplicity we assume that because of the binding and unbinding processes, the bulk complex diffusivity is of order smaller than the enzyme diffusivity and is therefore neglected.
3.2.3 Diffusion-reaction equation

We formulate the nondimensional reaction-diffusion equation by applying the law of mass action to the proposed kinetic model, and Fick’s law to the diffusion model:

\[
\begin{align*}
\partial_t s &= -\kappa \cdot s m + \lambda \cdot c \\
\partial_t m &= -s m + \frac{\lambda + \gamma}{\kappa} \cdot c + \delta \nabla \cdot (\nabla m) \\
\partial_t c &= \kappa \cdot s m - (\lambda + \gamma) \cdot c,
\end{align*}
\]

where \( s = S/\bar{S}, m = M/\bar{M}, c = C/\bar{S}, \) and \( \kappa = \bar{M}/\bar{S} \) are species concentration; bars denote the spatial average of the quantity at the surface at time zero, i.e. \( \bar{S} = l^{-2} \int \int S_i(x, y, z) dxdy \) and \( \bar{M} = l^{-2} \int \int M_i(x, y, z) dxdy \). Spatial coordinates are scaled by the length of the box, \( l = L = W \), and time is scaled by the molecular association time. Therefore the nondimensional length and time are \( x = \tilde{x}/l \) and \( t = k_i \tilde{S} \). The reaction rate constants and diffusivity are also scaled by molecular association time, which gives the last three nondimensional parameters \( \lambda = k_{-1}/(k_i \bar{S}), \gamma = k_2/(k_i \bar{S}) \) and \( \delta = D_M/(l^2 k_i \bar{S}) \). \( D_M \) is the effective enzyme diffusivity found in experiments \[31\]. The product concentration, \( p(x, y, z, t) \), is uncoupled from the other three species, and can be calculated directly from the mass conservation equation \( \partial_t p = -\partial_t (s + c) \). The initial conditions of this equation are given in the form

\[
\begin{align*}
s(x, y, z, t = 0) &= s_i(x, y, z) \\
m(x, y, z, t = 0) &= m_i(x, y, z) \\
c(x, y, z, t = 0) &= c_i(x, y, z),
\end{align*}
\]

where \( s_i(x, y, z) = S_i(x, y, z)/\bar{S}, m_i(x, y, z) = M_i(x, y, z)/\bar{M}, \) and \( c_i(x, y, z) = C_i(x, y, z)/\bar{S} \).

3.2.4 Mechano-chemical coupling

This chemical reaction model is coupled to the mechanical model in two aspects: first, the substrate degradation level is reflected by the product concen-
tration, as its stiffness decreases with increasing portion of denaturalized collagen fibrils, for example. As a first approach, we relate the Young’s modulus of the material to the product concentration linearly, i.e.

\[ E(x, y, z, t) = E_0[1 - \epsilon \cdot \alpha p(x, y, z, t)], \tag{3.4} \]

where \( \epsilon \) and \( \alpha \) are two arbitrary constants, which can be fitted to the experimental data [13]. \( \alpha \) links Young’s modulus with product concentration; \( \epsilon \) accounts for the inhomogeneity in the material. Note the similarity between this expansion and the one shown in Eq. (2.3); the solution we derived in the previous chapter is therefore applicable to this scenario as long as \( \alpha p(x, y, z, t) \sim \mathcal{O}(1) \). Second, it has been found that mechanical forces affect the catalytic reaction step, \( k_2 \). In particular, mechanical forces accelerate the catalytic reaction as illustrated by single molecule assays on MMP-I catalyzed collagen-I fibril proteolysis [17, 18]. The mechanistic interpretation of this discovery, brought up by Adhikari et al., is that the active site of MMP-I is too small to accommodate the collagen-I trimeric structure in a natural state. Therefore in prior to the proteolysis, the structure needs to be unwound and disrupted in order to fit into the enzyme active site edgewise like a ribbon. By changing the conformation of the collagen molecule, mechanical energy can be effectively used to overcome the energy barrier in order for the wound trimeric structure to unfold, and thus accelerate the denaturizing reaction. The quantitative behavior of this phenomenon is explained by Bell’s equation [32] or Kramer’s reaction rate theory [33], which suggested an exponential relations such as

\[ k_2(F) = k_{2,F=0} \cdot \exp\left(\frac{\beta \Delta E_m}{k_B T}\right), \tag{3.5} \]

where \( k_{2,F=0} \) is the dissociation rate constant for the stress-free conditions, \( \Delta E_m \) is the mechanical energy added to the system, and \( k_B T \) is the thermal energy in the environment. \( \beta \) is a proportional constant that needs to be fitted to experimental data. This model is confirmed experimentally [18, 17].

The solution of this reaction-diffusion equation coupled with elastostatic equation is sought numerically (we have the analytic solution for elastostatic equa-
Neumann-type boundary conditions are employed for each species to ensure zero flux at the boundaries. The initial condition is given by

\begin{align}
s_i(x, y, z) &= 1 \\
c_i(x, y, z) &= 0 \\
m_i &= \frac{1}{\bar{m}} \exp\left(-\frac{x^2}{2\nu^2} - \frac{y^2}{2\nu^2} - \frac{z^2}{2\nu^2}\right).
\end{align}

\(\bar{m} = 2\pi\nu^2[\text{erf}(0.5/(\sqrt{2}\nu))]^2\) is to normalize the distribution so that its integral on the surface is unity; \(\nu\) is a free parameter that has dimension of length. See FIG 1.2 for illustration of this setup. The numerical solver is written in Matlab with finite-difference scheme employed. The solution domain is five times the cell length in all direction. 64 grid points were placed in each direction. Neither increasing grid points nor increasing domain size affect solutions significantly.

Chapter 3, in part, is currently being prepared for submission for publication of the material. Jui-Hsien Wang; Juan Carlos del Álamo. The thesis author was the primary investigator and author of this material.
Chapter 4

Results

4.1 Verification and validation

We verify and validate the model in this section. As the classical explanations go, the difference between validation and verification can be respectively expressed by the query “Are you building the right thing?” and “Are you building it right?” We shall explain both of them in what follows.

We use the Michaelis-Menten kinetics with equilibrium approximation simplification to verify the numerical solver [26, 34]. When the dissociation reaction is rate-limiting, that is, $k_{-1} \gg k_2$, then the association and dissociation reactions are instantaneously in equilibrium, and the analytical expression to the reaction speed ($V_{rea} = dP/dt$) to the nonlinear reaction-diffusion equation (Eq. 3.2) is available:

$$V_{rea} = \frac{dP}{dt} = \frac{(k_2M_0)S}{K_1 + S},$$

where $K_1 = k_{-1}/k_1$, $S$, $M$, $P$ are the concentration of the substrate, enzyme, and product, respectively. Under the criterion, we compare the numerical and the theoretical solution in FIG. 4.1. After a short period of initialization, the association and dissociation reactions reach equilibrium and the numerical solution converges to the analytical ones. The simulation parameters are artificially chosen to match the assumptions of the equilibrium approximation. The diffusivity is eliminated in this simulation. The solution to the elastostatic problem is analytical and therefore
the verification simply involves the check of boundary conditions and satisfaction of the original equation.

\[
\text{Product reaction rate } \frac{d(P/S_0)}{d(t/(k_1 S_0))}
\]

\[\kappa = 1.00 \quad \lambda = 1.00 \quad \gamma = 0.05 \quad \delta = 0.00\]

**Figure 4.1**: Verification of the numerical solutions. The numerical solution of the reaction-diffusion equation is compared with the analytical ones obtained using the Michaelis-Menten approximation [26, 34].

As of the model validation, carefully conducted experiments are in need to justify the model. Many of these data are not available by the time this thesis is finished up, therefore we simply point out the parameters in the model that require further fitting to the experimental observations.

1. The perturbation parameter \(\epsilon\) of the Young’s modulus in Eq. (2.3).

2. The proportional parameter \(\alpha\) of how the product concentration affects the stiffness of the material (Young’s modulus), described in Eq. (3.4).

3. The proportional parameter \(\beta\) of the force-dependency on the catalytic reaction, given by Eq. (3.5).
4.2 Estimation of reaction constants

A series of studies on substrate specificity and measure of kinetic constants of the MMP family were performed since the early 1980s, concentrating on the proteolysis of collagen using MMP-1 [27, 35], MMP-2 [36, 37], MMP-8 [38, 35], and MT1-MMP [39]. The large body of work has shown that the enzyme-substrate affinities, independent of the collagenase types of interest, are similar across the different reaction substrates – human collagen type-I, type-II, and type-III. On the contrary, the catalytic rate, \( k_2 \), demonstrates sensitivity to the targeting substrates, including collagen type and species of substrate origin. For its simplicity and availability, we have selected to present the kinetic constants of MMP1-collagen(human) system:

Table 4.1: Reaction constants for the exemplary MMP1-collagen system found in the literatures.

<table>
<thead>
<tr>
<th>Units</th>
<th>Temp. ( ^\circ C )</th>
<th>( K_1 ) ( \mu M )</th>
<th>( k_1 ) ( \mu M^{-1} h^{-1} )</th>
<th>( k_{-1} ) ( h^{-1} )</th>
<th>( k_{2,F=0} ) ( h^{-1} )</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type-I Collagen</td>
<td>30</td>
<td>0.8</td>
<td>n.a.</td>
<td>n.a.</td>
<td>44</td>
<td>[35]</td>
</tr>
<tr>
<td>Type-I Collagen</td>
<td>25</td>
<td>0.8</td>
<td>n.a.</td>
<td>n.a.</td>
<td>53.4</td>
<td>[27]</td>
</tr>
<tr>
<td>Type-II Collagen</td>
<td>25</td>
<td>2.1</td>
<td>n.a.</td>
<td>n.a.</td>
<td>1.0</td>
<td>[27]</td>
</tr>
<tr>
<td>Type-III Collagen</td>
<td>30</td>
<td>1.7</td>
<td>n.a.</td>
<td>n.a.</td>
<td>350</td>
<td>[35]</td>
</tr>
<tr>
<td>Type-III Collagen</td>
<td>25</td>
<td>1.4</td>
<td>n.a.</td>
<td>n.a.</td>
<td>565</td>
<td>[27]</td>
</tr>
<tr>
<td>Synthetic Collagen</td>
<td>22</td>
<td>3.7</td>
<td>5.62</td>
<td>19.26</td>
<td>n.a.</td>
<td>[40]</td>
</tr>
</tbody>
</table>

n.a.: not available

In the table presented, Ottl et al. used synthetic collagen with the MMP-1(E200A) mutant to study the association and dissociation constants of the MMP1-collagen system [40]. The synthetic collagen (heterotrimer) they used is confirmed to have the identical structure as the collagen at 25\(^\circ\)C; in order to make the quantification of \( k_1 \) and \( k_{-1} \) easier, they used the MMP-1(E200A) mutant, which retains fully the substrate-binding affinity but is proteolytically inactive [40].

The collagen content of the substratum on which the cell migrate is another important parameter that will directly affect the reaction speed. A typical value of collagen content of the ECM structure in tumor tissue is below 5% [41, 42]. The
molecular weight of collagen is $\sim 300kDa$, which gives the molar concentration of the substrate, $\bar{S} \sim 200\mu M$, in this tissue. Other tissues are given as a reference, skeletal muscle 14%, corneal stroma 29%, subcutaneous tissue 21% [42].

As described in the previous chapter, we consider only the enzyme diffusivity. The diffusivity of MMP-1 is given by Collier et al., in the order of $\sim 0.8\mu m^2/s$ [30, 31]. For simplificity, in this study we employ this constant value throughout the domain, however, we note that it is possible to describe the diffusivity as a function of substrate concentration. Ogston et al. proposed a model to correct the diffusivity of spherical macromolecules embedded in porous material that is filled with cylindrical fibers [43]. Due to the hydrodynamic and steric effects, the effective diffusivity in gels is drastically lower than in free solution [44]. Although this effect is being ignored in the current model, we note that the numerical value reported by Collier et al., $D_M \sim 0.8\mu m^2/s$, is almost two orders of magnitude lower than the the diffusivity of MMP-1 in water, which is at the value $\sim 88\mu m^2/s$ (calculated using the molecular weight of MMP-1 54kDa) [45]. This effectively lowered diffusivity, as it is indeed measured with the presence of fibrilar structure, is justified to be directly applied to the study of the onset of cancer cell invasion, which is the rigor of our elastostatic model.

Finally, the nondimensional parameters used in the numerical simulation is given in Table 4.2.

**Table 4.2**: Nondimensional parameters used in the numerical simulations throughout the study if not specified otherwise.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\kappa$</td>
<td>$2.50 \times 10^{-1}$</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>$5.00 \times 10^{-3}$</td>
</tr>
<tr>
<td>$\gamma^*$</td>
<td>$8.90 \times 10^{-4} \sim 5.03 \times 10^{-1}$</td>
</tr>
<tr>
<td>$\delta$</td>
<td>$1.02 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

* force free
4.3 Analysis of a synthetic stress field

4.3.1 The synthetic stress field

In order to systematically study the dynamics of the system, we calculate the deformation field generated by a synthetic stress field applied on the surface of the substratum,

\[
\begin{align*}
\tau_{xx}(x, y, 0, t) &= 0 \\
\tau_{yy}(x, y, 0, t) &= 0 \\
\tau_{zz}(x, y, 0, t) &= T_0 \left(1 - \frac{r^6}{R^6}\right) \exp\left(-\frac{r^2}{R^2}\right),
\end{align*}
\]

where $T_0$ is the maximum stress, $R$ is the cell radius and $r$ is the distance to the cell center in polar coordinates. This stress field is plotted in FIG 4.2. The normal stress component employed here is qualitatively consistent with the traction force observed in the 3-dimensional cell migration and invasion experiments [21, 46, 47, 48, 49], and is tuned to be zero-averaged to be compatible with the elastostatic equation. The horizontal stresses ($\tau_{xx}, \tau_{yy}$) are set to zero as an assumption. We assume that the invading cell will align its cytoskeleton structure to the vertical direction and therefore results in higher vertical force compared to the horizontal ones [19, 21]. This assumption is justified by the experimental observation done by our collaborators. Adapted from Aung et al. [19], we show the response induced by a breast cancer cell (cell line: MDA-MB-231) during a vertical invasion experiment can be well approximated by Eq. 4.2. Moreover, subfigure (c), which plotted the DIC images superimposed by the traction stresses in x-y plane and the traction stresses in x-z plane, shows that the normal stresses measured during the invasion are much higher than the horizontal stresses. The ideal stress boundary condition we imposed are therefore of biologically relevance.
Figure 4.2: Synthetic stress field on the surface of the substratum. Top row: the applied stress on the surface ($z = 0$) of the substratum. Color represents the magnitude of the stress in Pascals. Bottom row: the center cut-plane of the applied stress on the surface ($y = 0, z = 0$) of the substratum. Parameters: $E_0 = 400(Pa), T_0 = 150(Pa)$
4.3.2 On strain-softening material

Several biomaterials are identified to have nonlinear mechanical properties (illustration see FIG. 4.4). Cross-linked collagen network and many of the \textit{in vivo}
tissues demonstrate strain-stiffening properties, while Matrigel, a popular choice of material for cell culture and *in vitro* cell locomotion studies, demonstrates strain-softening properties [50]. In addition to this mechanical yielding effect, permanent, irreversible substrate degradation due to the enzymatic proteolysis can also cause a similar response [13]. As the current traction force cytometry methods (TFM) is linear, there is no analytical or numerical tools to quantify this nonlinear response. By applying the analytical method we’ve developed in chapter 2, we can now be starting to study this effect.

![Stress-strain plot of prototypical 1-D nonlinear materials.](image)

**Figure 4.4: Stress-strain plot of prototypical 1-D nonlinear materials.** Red dashed-line: typical strain-softening material; Green dashed-dot-line: typical strain-stiffening material

As a demonstration, we apply the synthetic stress field defined in Eq. 4.2 on a strain-softening material, the Young’s modulus of which is characterized by:

\[
E = E_0 \left[ 1 - 0.25|\nabla \mathbf{u} + \nabla \mathbf{u}^T| \right].
\] (4.3)

For this constitutive relation, perturbation constant, \( \epsilon \), is chosen to be 0.5 and the perturbation function, \( f(x, y, z) \), is chosen to be lowered by the magnitude of normal strain, and hence \( f = -0.5(\nabla \mathbf{u} + \nabla \mathbf{u}^T) \). The selection of these two constants are to serve the sole purpose of demonstration; they are arbitrary and can be fitted to different experimental materials and situations. The leading order, first order, and the total deformation in \( x, y, \) and \( z \) directions are shown in FIG. 4.5.
Figure 4.5: Deformation field on the surface of a strain-softening material. (a): leading order; (b): first order; (c): total deformation with $\epsilon = 0.5$. Color represents the vertical deformation, $w$; vectors show qualitatively the magnitude and direction of horizontal deformation, $u$ and $v$.

4.3.3 On continuously degrading material: study using the mechano-chemical model

The enzymatic proteolysis causes continuous degradation on the material. We model the dynamics of this time-evolving system using the mechano-chemically coupled system constructed in the previous chapters. The stress boundary conditions are kept constant with time as a demonstration that the cell does not need to do more mechanical work when the proteolysis effects are presented. The numerical model parameters listed in Table 4.2 are used throughout the section if not specified otherwise.
Evolving deformation field on different substratum within given period of time

Table 4.1 shows that the catalytic reaction rates, $k_2$, of MMP-1 enzyme are quite different for human collagen type-I ($k_2 = 53.4h^{-1}$ or $\gamma = 4.75 \times 10^{-2}$), type-II ($k_2 = 1h^{-1}$ or $\gamma = 8.9 \times 10^{-4}$), and type-III ($k_2 = 565h^{-1}$ or $\gamma = 5.03 \times 10^{-1}$) substrates. We first examine how do these values affect the deformation profile and substrate degradation. We observe the period of 1 minute with 0.5 seconds interval, as the model is most suitable for the study of invasion onset. FIG. 4.6 shows the evolution of deformation profile on the surface of the substratum, and the average concentration of the four chemical species developing with time. There are two reasons why this deformation profile is higher than a few microns per hour that is suggested by the experiments [41]: (1) we assumed that the enzyme and mechanical forces are readily applicable at time zero. By this assumption we are neglecting for example the time for enzyme expression, enzyme activation, adhesion site formation, aligning the cytoskeleton structure and so forth. Although these effects contribute to the overall time-lag in experiments, they have little effect on the mechano-chemical coupling. The qualitative feature of the system’s dynamics is of most interest in this study. (2) because of the above reason, in FIG. 4.6 we are showing the first order, vertical deformation before scaling. That is, we still have the flexibility to choose over different values of $\epsilon$ to align our simulation results to experimental observations.

As expected, the first order deformation profile is closely related to the catalytic reaction constant as it affects the degradation speed (zeroth order remains the same despite the change in the first order). The higher the catalytic rate the faster the vertical deformation builds up. The difference of the substrate specificity is directly reflected on the evolution of the deformation profile and is significant. This high sensitivity indicates that the $k_2$ reaction is not only the rate-limiting process of the chemical reaction, but also the rate-limiting and a necessary process in the invasion cascade.
To estimate the position of the cell center, we average the first order vertical deformation within the cell radius ($r \leq R$). The result is plotted with respect to time in FIG. 4.7. The initial slope of the cell center position is found to be positively related to $k_2$: the higher the catalytic rate, the faster the cell center moves. The cell center position slows down when placed on collagen type-III substratum at the slow time scale. This is a result of positive deformation that is also building up. From FIG. 4.6(c), we found that the positive deformation is increasing around the cell perimeter ($X/D \sim 0.5$) after merely 0.2 minutes. At this time, the excessive amount of enzymes that are diffusing through the entire matrix start to degrade the support of the cell, that is, where the cell develops positive deformation. Once the stiffness of these supports is lowered, the positive deformation increases and the cell is pulled back by itself. This is a curse by the physics underlying the vanishing inertia effects; the cells have to break the symmetry in order to move downwards. We showed that excessive, uncontrolled degradation, especially at the perimeter of the cell where the positive deformation builds up, can slow down the invasion process because it is also destroying the scaffold on which the cell can adhere.
Figure 4.6: First order deformation field on the surface of a continuously degrading material. (a): collagen type-I matrix; (b): collagen type-II matrix; (c): collagen type-III matrix. Collagen type-I, type-II, and type-III matrices are distinguished by their catalytic reaction rate, shown in Table 4.1. Left panel: time development of the surface vertical deformation at the center cut-plane \((y = 0, z = 0)\). Color represents the simulation time (unit: minutes). Right panel: time development of the chemical species. The magnitude of Young’s modulus, \(|E|\), is calculated using \(\epsilon = 0.5\) and \(\alpha = 1\) (see Eq. 3.4). \(s, m, c, p\) are respectively the nondimensional concentration of substrate, enzyme, substrate-enzyme complex, product.
Figure 4.7: Position estimation of the cell center. Collagen type-I, type-II, and type-III matrices are distinguished by their catalytic reaction rate, shown in Table 4.1.

Effects of strain-mediated catalytic reaction rate

Next we examine the effects of the strain-mediated catalytic reaction rate on all three substrates. The equation we used for determining the catalytic reaction rate is given by Eq. (3.5). Although this relation is qualitatively suggested by the experiments [32, 33, 17, 18], it is interesting to see how the value of $\beta$ affects the development of deformation field on the substratum. To this end, we study the time required for different values of $\beta$ that results in substratum deformation more than $0.5\mu m$, i.e. 10% of the model cell radius. The result is shown in FIG. 4.8. It is observed that the required time is shorter for higher $\beta$, for that the mechanical force has stronger effect on the catalytic rate. However, the deformation is rather insensitive to the choice of $\beta$, especially for higher $k_2$ matrices, such as the prototypical collagen type-III matrix; the changes are shown to be very mild. This result suggests that, for these matrices, the rate-limiting process might be shifted from the catalytic reaction to the formation of enzyme-substrate complex, which is controlled by the values of $k_1$ and $k_{-1}$ but not $k_2$; for these matrices the strain-mediated catalytic reaction is thus not important. Also, we note that because the
effective catalytic rate is being accelerated by the mechanical strain, the classical Michaelis-Menten kinetics breakdown earlier because the assumption $k_2 \ll k_{-1}$ is not valid for substratum subjected to high mechanical strain [26].

![Graph showing time required for substratum deformation for different values of β.](image)

**Figure 4.8**: Time required for 0.5μm substratum deformation for different values of β. Collagen type-I, type-II, and type-III matrices are distinguished by their catalytic reaction rate, shown in Table 4.1.

**Effects of enzyme diffusivity**

Invading cancer cells are known to be capable of regulating the enzymatic proteolysis in ways including cell surface tethered enzyme secretion, in situ enzyme activation, and molecular diffusion [30, 31]. The continuum mechanics framework, in which we are developing our model, is capable of representing these effects by an effective diffusivity. For example, membrane-tethered type-1 MMP, MT1-MMP, is an enzyme that is bound to the cell surface when activated. This enzyme can be considered as immobile (or has very small mobility) in the system. By eliminating the enzyme diffusivity, $\delta$ in Table 4.2, we can virtually model an invasive cell that is expressing MT1-MMP. In the physical point of view, to make the best use of the activated enzymes to promote the invasion (the deformation), is it a better
strategy to eliminate the enzyme diffusivity, or to let it diffuse to where the enzyme concentration is lower? This appears to be a fascinating question, which our model might be able to provide some insights to. In this section we discuss the enzyme diffusivity.

We compare the simulation cases with and without diffusivity in FIG. 4.9 for five minutes of simulation time. The time development of first order vertical deformation (top row) and Young’s modulus (bottom row) are shown at the center cut-plane at the surface of the substratum \((y = 0, z = 0)\). We found that by knocking out the diffusivity, the substrate degradation is faster than the case with diffusivity; a possible explanation for this phenomenon is that the diffusing enzymes lowers the colocalized enzyme concentration, i.e. these enzymes diffuse to places where substrate deformation is relatively unimportant and impairs the creation of deformation profile. We therefore examine the vertical cut-plane of the substrate \((x = 0, y = 0)\). In FIG. 4.10, the Young’s modulus at this plane plotted with time shows that the degradation of the diffusive case reaches deeper into the substrate with time. For a certain total amount of enzymes available, diffusivity is therefore impairing the local degradation speed at the location where the cell lies, and the vertical deformation profile it can develop. More investigation on diffusivity should be conducted in two directions to further analyze this phenomenon: (1) through experimental comparison; (2) through the application of the nonuniform diffusivity such as those in ref. [43, 44].
Figure 4.9: Effects of diffusivity on deformation and substrate degradation (horizontal cut-plane). Top row: Kymograph of first order, vertical deformation at the cut-plane \( y = 0, z = 0 \). Color represents the magnitude of the deformation. Bottom row: Kymograph of Young’s modulus at the same cut-plane. Color represents the magnitude of the Young’s modulus. \( \epsilon = 0.5 \).
Parametrized study of the mechanical forces, amount of total enzyme concentration, and the enzyme release

As demonstrated in the last section, the mechanical and chemical factors affect the model cell invasion, individually or jointly. A natural question arises: what is the best – or in some regards the worst – combination of mechanical force and enzyme release, including its amount and distribution, that will result in the greatest invasion efficiency? To answer this question, we perform a parametrized study on three quantities: the magnitude of mechanical forces applied by the cell, total enzyme amount that is initially released in the system, and initial distribution of the enzyme concentration.

In FIG 4.11, we plot the penetration depth at $t = 20$ on the surface of the substrate with respect to the measure of mechanical force, $\xi = T_0/E_0$, and the amount of total enzyme concentration, $\kappa = \bar{M}/\bar{S}$ (refer back to section 3. $\xi$ is a non-dimensional quantity that indicates the relative stiffness: if $\xi$ is higher, the cell is in a relatively softer environment and vice versa. At low enzyme concentration ($\kappa < 0.2$), the penetration depth is very sensitive to the total enzyme amount $\kappa$. 

Figure 4.10: Effects of diffusivity on Young’s modulus (vertical cut-plane). Kymograph of Young’s modulus at the cut-plane $x = 0, y = 0$. Color represents the magnitude of the Young’s modulus. $\epsilon = 0.5$. 

(a) without diffusivity
(b) with diffusivity
but not the mechanical forces; at high enzyme concentration ($\kappa \geq 0.2$), mechanical forces dominate the behavior and $\kappa$ becomes less important. White dashed-line in FIG 4.11(a) and (b) shows the optimum $\kappa$ for a specific value of $\xi$ that will result in maximum penetration depth. Interestingly, this value decreases with increasing mechanical force, meaning that in a high mechanical force regime, increasing enzyme secretion/activation lower the invasion performance. An explanation of this can be given by examining the two designated cases marked by “$X$” and “$O$” on the figure. Excessive enzyme concentration (marker “$X$”) degrades the entire matrix faster; as shown in FIG 4.12, while the center stiffness has been lowered to the same value, excessive enzyme also degrades the region $X/D \sim 0.5$ where the cell is pulling up in the vertical direction. As a consequence, the positive deformation around that site is slightly higher, counterbalancing the negative deformation and result in lower overall performance. Physically speaking, the cell destroys its own support by releasing excessive amount of enzyme and therefore impairs its capability to break the symmetry in the first order. The average penetration depth, shown in FIG 4.11(b), demonstrates the same trend. Young’s modulus are plotted with respect to $\xi$ and $\kappa$ in FIG 4.11(c)-(d) for reference.

In FIG 4.13, we plot the penetration depth at $t = 20$ on the surface of the substrate with respect to the same parameter $\xi$, and the parameter $\nu$ that controls the spatial variation of the initial enzyme distribution (for definition refer back to Eq. 3.8). $\nu$ quantifies how spread out is the initial enzyme distribution (with a fixed total amount): if $\nu$ is higher, the activated enzyme are more spreadout and vice versa. We study the cases where $\nu$ ranges from $1/3$ to 3 times of the cell radius. In general, increasing mechanical forces will result in higher penetration depth. We note that the optimum values of $\nu$ gradually increase with increasing $\xi$; this result is suggesting that for the cells that are not able to generate a great deal of force, concentrating the enzyme secretion at a very small area will be more efficient, however, for cells that can generate greater mechanical force, it will be better to distribute the activated enzyme within the cell radius. The average penetration demonstrates the same trend, shown in FIG 4.13(b). Young’s modulus are plotted with respect to $\xi$ and $\nu$ in FIG 4.13(c)-(d) for reference.
Figure 4.11: The effect of mechanical force and total enzyme amount on penetration depth and Young’s modulus. Penetration depth and Young’s modulus after $t = 20$ on the surface of the substrate with respect to the measure of mechanical force, $\xi = T_0/E_0$, and the amount of total enzyme concentration, $\kappa = \bar{M}/\bar{S}$. (a): maximum penetration depth; (b): average penetration depth on the surface. Color represents the magnitude of the depth in terms of $w_1/D$. (c): remaining stiffness at the severest point (cell center); (d): average remaining stiffness on the surface. Color represents the remaining stiffness (%). White dashed-line: position of maximum penetration depth for each $\xi$. Green markers: selected cases for the study of excessive enzyme, see FIG 4.12. Other simulation parameters: $E_0 = 500(Pa)$, $T_0 = E_0 \cdot \xi$, $\lambda = 0.25$, $\gamma = 0.2$, $\delta = 0$. 
Figure 4.12: Mechanical force and total enzyme amount: case-study. Vertical deformation (left) and remaining stiffness distribution (right) at the center cut-plane on the surface ($y = 0, z = 0$) after $t = 20$. Red line: $\xi = 5.25$, $\kappa = 0.25$ (marker “X” in FIG 4.11); blue line: $\xi = 5.25$, $\kappa = 1.12$ (marker “O” in FIG 4.11). Other simulation parameters: $E_0 = 500(Pa)$, $T_0 = E_0 \cdot \xi$, $\lambda = 0.25$, $\gamma = 0.2$, $\delta = 0$. 
Figure 4.13: The effect of mechanical force and initial enzyme distribution on penetration depth and Young’s modulus. Penetration depth and Young’s modulus after $t = 20$ on the surface of the substrate with respect to the measure of mechanical force, $\xi = T_0/E_0$, and the standard deviation of the initial enzyme distribution, $\kappa = \bar{M}/\bar{S}$. (a): maximum penetration depth; (b): average penetration depth on the surface. Color represents the magnitude of the depth in terms of $w_1/D$. (c): remaining stiffness at the severest point (cell center); (d): average remaining stiffness on the surface. Color represents the remaining stiffness (%). White dashed-line: position of maximum penetration depth for each $\xi$. Other simulation parameters: $E_0 = 500(Pa)$, $T_0 = E_0 \cdot \xi$, $\kappa = 0.25$, $\lambda = 0.25$, $\gamma = 0.2$, $\delta = 0$.

Chapter 4, in part, is currently being prepared for submission for publication of the material. Jui-Hsien Wang; Juan Carlos del Álamo. The thesis author was the primary investigator and author of this material.
Chapter 5

Discussions

We start by introducing a novel method to calculate the deformation profile that is induced by stresses on a nonlinear, inhomogeneous material. This theory is extended from the Fourier Traction Force Cytometry method (TFM), which was developed previously in our group. Analytical solution of this problem is derived using the regular perturbation method. By the asymptotic expansion on Young’s modulus and the deformation vector, our general theory framework can be easily adapted to the study of durotaxis, which is characterized by the linear gradient of material stiffness, and to the precise response quantification on Matrigel and Collagen, which are respectively characterized by the stress-softening and stress-stiffening material properties, etc. Owing to the nature of the perturbation method, this result is more rigorous for small nonlinearity and inhomogeneity, yet this constraint might be removed using multiscale method as a future work. Because of the flexible mathematical framework, we can easily apply the result from Chapter 2 to the construction of nonlinear traction force microscopy method. This can be done by changing the boundary conditions at the surface and the mathematical derivation is similar. As all the current TFM method is linear, this capability opens a new window of opportunities on the study of cell mechanics.

To construct a theoretical representation of protease-dependent cancer cell invasion, we then combined this analytical tool with a chemical model that accounts for the dynamics of the system, namely the enzyme kinetics and enzyme
diffusion. A two-step kinetics scheme is applied with a simple molecular diffusion to formulate the system of governing equations. We solved these equations numerically using customized finite-difference solver written in Matlab. Without losing the generosity of the model, realistic parameters obtained from cancer cell invasion experiments are applied. The mechanical and chemical model are coupled under the considerations that, (1) the concentration of the denaturized substrate, such as Collagen, can be related to the Young’s modulus of the material; (2) the catalytic reaction is accelerated by the presence of strain energy, as found in the recent single molecule assays [17, 18].

This mechano-chemical model is used to study a synthetic stress field that is consistent with experimental observations. The responses from the leading order and the first order are shown with the evolution of time. It is found that the evolution of the deformation profile, or the “invasion speed“, increases with the increasing catalytic reaction rate. The excessive amount of enzymatic proteolysis at the peripheral of the cell can destroy the structural support and slow down the deformation speed. Therefore the effects of uniform diffusivity are studied. It is found that vertical enzyme diffusion might also impair the deformation speed as the total concentration of the enzyme in the system is conserved. Finally, we studied the effects of mechanical forces, total enzyme concentration, and the enzyme release systematically. This parametrized study indicates that for a system of insufficient colocalized enzymes, the deformation is very sensitive to the amount of presented enzymes. Above a critical value, the amount of presented enzymes has little effect on the deformation magnitude. We found that the optimum amount of enzyme release, which will give maximum deformation, decreases with increasing force, suggesting the existence of a possible minimum energy consumption scheme for the cancer cell to invade.

The future work aligns in several directions: (1) we will continue to refine our model accuracy by fitting it to experiments; (2) the non-uniform enzyme diffusivity could be introduced to further quantify the effects on deformation; (3)
an additional forcing term in the reaction-diffusion equation can be included to account for the MMPs potentially secreted by the surrounding endothelial cells during the invasion; (4) the presence of near-by-cells can be studied by changing the boundary conditions of reaction-diffusion equation into periodic ones; (5) polymer mesh size can potentially have an effect on the variation of the Young’s modulus and can be included using models conforming to the continuum hypothesis; (6) linear stability analysis can be performed to get further insights on the importance of each parameters and to compare with the numerical simulation. Having this mechano-chemical model in hand, we will continue to explore the interplay among mechanical forces, enzymatic substratum degradation, and cell invasion in the physical and mathematical point of views.

Chapter 5, in part, is currently being prepared for submission for publication of the material. Jui-Hsien Wang; Juan Carlos del Álamo. The thesis author was the primary investigator and author of this material.
Appendix A

Explicit expression for the elastostatic problem

\[ U_{mn}(z) = \begin{bmatrix} -\frac{\alpha^2(z+h) \cosh(k(z+h))}{4k^2(\sigma-1)} + \frac{\left(-3k^2+4k^2\sigma-\beta^2\right) \sinh(k(z+h))}{4k^3(\sigma-1)} \\ \frac{\alpha \beta (-k(z+h) \cosh(k(z+h)) + \sinh(k(z+h)))}{4k^3(\sigma-1)} \\ \frac{i\alpha (z+h) \sinh(k(z+h))}{4k(\sigma-1)} \end{bmatrix} \] (A.1a)

\[ V_{mn}(z) = \begin{bmatrix} -\frac{\beta^2(z+h) \cosh(k(z+h))}{4k^2(\sigma-1)} + \frac{\left(-3k^2+4k^2\sigma-\alpha^2\right) \sinh(k(z+h))}{4k^3(\sigma-1)} \\ \frac{i\beta (z+h) \sinh(k(z+h))}{4k(\sigma-1)} \\ \frac{i\sinh(k(z+h))(z+h)\alpha}{2k(2\sigma-1)} \end{bmatrix} \] (A.1b)

\[ W_{mn}(z) = \begin{bmatrix} \frac{i\sinh(k(z+h))(z+h)\beta}{2k(2\sigma-1)} \\ \frac{(z+h) \cosh(k(z+h))}{2(2\sigma-1)} + \frac{(4\sigma-3) \sinh(k(z+h))}{2k(2\sigma-1)} \end{bmatrix} \] (A.1c)
\[ U_{mn}(z) = \begin{bmatrix} \cosh(k(z + h)) - \frac{1}{2} \frac{\alpha^2(z+h) \sinh(k(z+h))}{k(2\sigma-1)} \\ -\frac{1}{2} \frac{\alpha \beta(z+h) \sinh(k(z+h))}{k(2\sigma-1)} \\ -\frac{1}{2} \frac{\alpha \beta(z+h) \sinh(k(z+h))}{k(2\sigma-1)} \\ -\frac{1}{4} \frac{i\alpha (-k(z+h) \cosh(k(z+h))+\sinh(k(z+h)))}{k(\sigma-1)} \\ -\frac{1}{4} \frac{i\beta (-k(z+h) \cosh(k(z+h))+\sinh(k(z+h)))}{k(\sigma-1)} \end{bmatrix} \] \quad (A.2a)

\[ V_{mn}(z) = \begin{bmatrix} \cosh(k(z + h)) - \frac{1}{2} \frac{\beta^2(z+h) \sinh(k(z+h))}{k(2\sigma-1)} \\ -\frac{1}{2} \frac{i\beta (-k(z+h) \cosh(k(z+h))+\sinh(k(z+h)))}{k(2\sigma-1)} \\ -\frac{1}{2} \frac{i\beta (-k(z+h) \cosh(k(z+h))+\sinh(k(z+h)))}{k(2\sigma-1)} \\ -\frac{1}{4} \frac{\beta (k(z+h) \cosh(k(z+h))-\sinh(k(z+h)))}{k(\sigma-1)} \\ -\frac{1}{4} \frac{\beta (k(z+h) \cosh(k(z+h))-\sinh(k(z+h)))}{k(\sigma-1)} \end{bmatrix} \] \quad (A.2b)

\[ W_{mn}(z) = \begin{bmatrix} \cosh(k(z + h)) + \frac{1}{4} \frac{k(z+h) \sinh(k(z+h))}{\sigma-1} \\ \frac{1}{4} \frac{i\alpha (k(z+h) \cosh(k(z+h))-\sinh(k(z+h)))}{k(\sigma-1)} \\ \frac{1}{4} \frac{i\beta (k(z+h) \cosh(k(z+h))-\sinh(k(z+h)))}{k(\sigma-1)} \end{bmatrix} \] \quad (A.2c)

\[ \mathcal{H}_{\alpha\beta}(E, \sigma) = \frac{E}{2(1+\sigma)} \begin{bmatrix} 0 & 0 & i\alpha & 1 & 0 & 0 \\ 0 & 0 & i\beta & 0 & 1 & 0 \\ 2i\alpha\sigma & 2i\beta\sigma & 0 & 0 & 2(1-\sigma) & \frac{1}{1-2\sigma} \end{bmatrix} \] \quad (A.3a)
\[
\hat{G}_{11}^* = \frac{((z + h) \beta^2 - k^2 (z + h) - k (4 \sigma - 3)) e^{-(z+h)k}}{k (4 \sigma - 3)} - \frac{(8 \alpha' \sigma^2 + (4 \beta' k (z - h) + 8 \beta^2 - 4 k^2 (z - h) - 12 k^2 \sigma) e^{-(z+h)k}}{k^2 (4 \sigma - 3)} - \frac{(-2 \beta^2 + 5 k^2 - 2 k^2 h \sigma^2 + 3 \alpha^2 k (z - h)) e^{-(z+h)k}}{k^2 (4 \sigma - 3)}
\]

\[
\hat{G}_{22}^* = + \frac{\alpha' k (z + h) e^{-(z+h)k}}{k^2 (4 \sigma - 3)} + \frac{\alpha' \beta k (z + h) e^{-(z+h)k}}{k^2 (4 \sigma - 3)}
\]

\[
\hat{G}_{13}^* = + \frac{\alpha' \beta k (z + h) e^{-(z+h)k}}{k^2 (4 \sigma - 3)}
\]

\[
\hat{G}_{31}^* = + \frac{\alpha' \beta k (z + h) e^{-(z+h)k}}{k^2 (4 \sigma - 3)}
\]

\[
\hat{G}_{21}^* = + \frac{\alpha' \beta k (z + h) e^{-(z+h)k}}{k (4 \sigma - 3)}
\]

\[
\hat{G}_{32}^* = + \frac{\alpha' \beta k (z + h) e^{-(z+h)k}}{k (4 \sigma - 3)}
\]

\[
\hat{G}_{33}^* = + \frac{\alpha' \beta k (z + h) e^{-(z+h)k}}{k (4 \sigma - 3)}
\]

\[
\hat{G}_{22}^* = + \frac{\alpha' \beta k (z + h) e^{-(z+h)k}}{k (4 \sigma - 3)}
\]

\[
\hat{G}_{31}^* = + \frac{\alpha' \beta k (z + h) e^{-(z+h)k}}{k (4 \sigma - 3)}
\]

\[
\hat{G}_{32}^* = + \frac{\alpha' \beta k (z + h) e^{-(z+h)k}}{k (4 \sigma - 3)}
\]

\[
\hat{G}_{33}^* = + \frac{\alpha' \beta k (z + h) e^{-(z+h)k}}{k (4 \sigma - 3)}
\]
References


